1977

The patterns for glycogen utilization during work overload in the isolated perfused rat heart.

Kenji Alan. Kenno
University of Windsor

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE PATTERNS FOR GLYCOGEN UTILIZATION DURING WORK OVERLOAD IN THE ISOLATED PERFUSED RAT HEART

by

Kenji Alan Kenno

A Thesis
submitted to the Faculty of Graduate Studies
through the Faculty of
Human Kinetics in Partial Fulfillment
of the requirements for the Degree
of Master of Human Kinetics at
The University of Windsor

Windsor, Ontario, Canada

1976

To Mom and Dad

ACKNOWLEDGEMENTS

The author is indebted to Dr. F. Cerny, Dr. P. Taylor and Dr. B. Virgo for invaluable guidance and assistance throughout the investigation.

Special thanks are extended to M. Baillargeon at the University of Windsor computer centre.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

During relatively low workloads well oxygenated hearts preferentially oxidize exogenous free fatty acids to maintain ATP levels (4,18,23;26,30,42). As the heart performs more work there appears to be a coordinated increase in the uptake (5,15,16,21-23) and oxidation (5,15,21-23) of both glucose and free fatty acids, as well as utilization of endogenous lipids and glycogen.

At rest, carbohydrates are stored in the rat myocardium in the form of glycogen, at relatively constant levels (10,40). It has been also shown that cardiac glycogen can be mobilized to maintain myocardial performance during anoxia (38,39) and substrate free perfusion (13,18,24,27,32,41) as well as during elevated workloads (18,19,22,27). This mobilization of glycogen also appears to be dependent upon the availability of alternative substrates (18-20,24,27,46-48) and the duration of the workload (2,40).

Early investigations (2,10,33,35,36), using exercised animals showed that the level of glycogen decreased over time, but did not find a consistent pattern of glycogen depletion over time. More recently, studies

using the perfused heart attempted to determine if distinct glycogen depletion patterns exist. Using substrate free perfusates, which force the heart to utilize its endogenous substrates, in particular, glycogen; the rate of glycogen depletion was forced to increase as perfusion pressure was raised (18,19,24,27). The addition of glucose to the perfusate reduced the rate of glycogen depletion seen during substrate free perfusions, but failed to completely inhibit glycogen breakdown at the higher workloads (18,19,22,27). The failure of exogenous glucose to inhibit glycogen breakdown may be that glucose transport is a rate limiting step in glucose oxidation (14,15,22,28), especially at higher workloads (15,22,28).

The concentration of glucose has been shown to affect glycogen depletion; Opie et al (32), using 1.25mM glucose reported glycogen depletion during low perfusion pressures, suggesting the exogenous glucose concentration was inadequate to maintain glycogen levels. A similar relationship has been suggested by Neely and coworkers (28) who suggested that 5mM glucose was unable to saturate glucose transport, thus stimulating glycogen breakdown unless there was a presence of insulin, fatty acids and/or the concentration of the glucose was raised.

In contrast, at a low level of work, the addition of either physiological (11,15,47,48) or hyperglycemic (15,32) concentrations of glucose were able to prevent glycogenolysis in the perfused rat heart. Furthermore,

Dhalla et al (7), using a 8mM glucose medium and Opie et al (32), continually increasing the glucose concentration, have shown that following a 10 minute substrate free prewash, there was actually a net synthesis of glycogen; adding considerably to the confusion of how exogenous glucose affects glycogen stores.

It appears that the utilization of exogenous glucose and endogenous glycogen at low and high workloads might depend upon the interaction of these substrates and others (endogenous lipids, lactate) which may contribute to the total energy production.

It is important to note, however, that, the rates of substrate utilization and interaction of substrates, including glycogen, during the extreme conditions of hyperglycemia and substrate free perfusions at varying workloads, may be altered, from those rates and interplay seen with physiological glucose concentrations. It is important to identify the patterns of glycogen depletion associated with increased work in the presence of physiological glucose concentrations; this may give a better indication of the metabolic factors that affect the utilization of glycogen.

Hypothesis

It was hypothesized that increasing the workload in the heart would displace the pattern of glycogen utilization and that under 3 workloads, 3 different

patterns of glycogen utilization would occur.

Previous work overload experiments using varying substrates (18,19,22,24,27) support this hypothesis. These studies have not, however, identified the pattern of glycogen depletion under 3 workloads with physiological glucose concentrations. The purpose of my study was to measure the myocardial glycogen concentration at regular intervals over 1 hour of perfusion, and thus determine the depletion of glycogen over time. Moreover, I expected the results to give an indication of metabolic interplay affecting these patterns of glycogen depletion.

It was further hypothesized that: a low workload would probably cause little or no glycogenolysis. Evidence supporting this are investigations using physiological glucose concentrations showing no glycogen depletion at low workloads (11,15,47,48). In addition, it was suggested that as perfusion time was prolonged, glycogen would be utilized. This view was based on pilot studies and on the assumptions that endogenous lipids would be depleted (5,6,18,26,41) and that glucose transport would be rate limiting (14,22,32).

At a moderate workload (90mmHg), it was hypothesized little glycogen depletion would be expected, as previous experiments (18,24,27) using 15mM glucose at a fairly high perfusion pressure (100mmHg) reported little glycogen depletion. Glycogen utilization would be expected following this sparing of glycogen, due to a

possible decreased glucose concentration (14,22,32) and potential depletion of endogenous lipids (5,6,18,26,41) during the initial period of perfusion. Glycogen depletion may then decrease, indicating that alternative substrates (i.e., glucose, lactate) were being used as a source of energy.

Thirdly, it was hypothesized that at a higher workload the pattern of glycogen depletion would be very rapid at the outset, as indicated from the literature (18,19,27) and pilot studies; as glycogen would be the most available energy substrate while aerobic metabolism was adjusting to meet the energy demands of the workload. Following this adjustment, glycogen utilization would be progressively slowed as perfusion time was prolonged. This may be explained by the combined utilization of other substrates (glucose, lactate, lipids) by the myocardium.

CHAPTER II

METHODS

It was hypothesized that changes in the workload of the heart perfused with physiological concentrations of glucose, would alter the pattern of glycogen utilization and that under 3 workloads, 3 different patterns of glycogen utilization would occur.

To test the hypothesis, it was decided to use the Langendorff perfusion apparatus with Krebs-Henseleit bicarbonate buffer containing physiological concentrations of glucose. The glycogen levels were determined at 15 minute intervals during 1 hour of perfusion at 60, 90 and 120mmHg perfusion pressure.

Animal Care and Handling

Male Wistar rats (Woodlyn Laboratories, Guelph) weighing 456±25 (range 398 to 501gm) were housed 1 or 2 per cage in a Hotpack Environmental Chamber (model 682) at 22°C under 12 hours of light per day (0700-1900 hr). The animals were maintained on Purina laboratory chow and tap water ad libitum.

Preparation of the Heart for Perfusion

All the experiments were done between the hours

of 0800-1700 hours. All the animals were anaethetized with Nembutal (30mg/kg i.p.) just before the abdominal cavity was opened by making a midline incision with scissors just below the rib cage. To prevent the blood from clotting in the coronary vessels, 200 units of heparin were injected into the inferior vena cava. After 15 seconds, the thoracic cavity was opened by making lateral cuts along each side of the rib cage; the diaphragm was transected and the heart removed and placed into ice cold .9% saline to arrest the contractions. The heart was then removed from the saline with fine tipped forceps and the aorta slipped onto a grooved, stainless steel cannula (12 gauge) of a modified Langendorff perfusion apparatus (Figure 1) (14,25) and secured with a silk ligature. The time from excision to the cannulation of the heart took 30-45 seconds.

Heart Perfusion

A 10 minute non-recirculating washout perfusion was begun immediately by unclamping the tubing from a reservoir 75 centimeters above the heart. This preliminary perfusion served to wash out any blood in the coronary vessels, equilibrated the glucose concentration in the interstitial fluid with that in the perfusate, allowed stabilization of cardiac rate and force and allowed recovery of the heart from the period of anoxia associated with excision and cannulation.

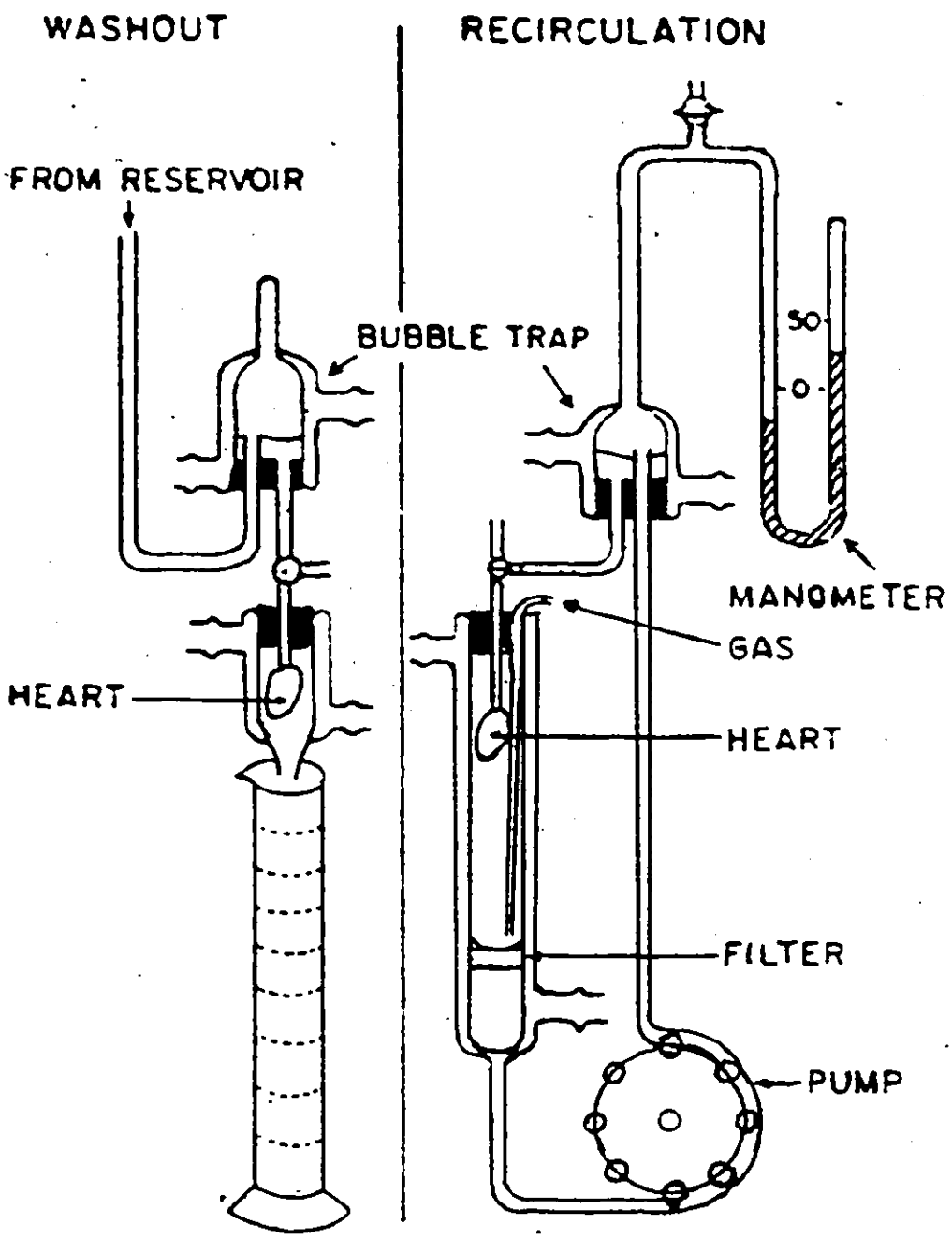

FIGURE 1. DIAGRAMMATIC REPRESENTATION OF THE PERFUSION APPARATUS (14)

The perfusate was a Krebs-Henseleit bicarbonate buffer containing the following salts in millimoles per liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; Mg_2SO_4, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; NaEDTA, 0.5; and glucose 5.5. The perfusate was maintained at 37°C and oxygenated with 95%, O_2: 5%, CO_2 gas mixture saturated with water at 37°C.

During this preliminary washout perfusion the pulmonary artery was cut to eliminate possible back pressure; also any connective tissue, thymus or lung attached to the heart was removed. The heart was then transferred to a water jacketed glass chamber maintained at 37°C. The tubing from the reservoir was clamped, while simultaneously opening the tubes leading to the peristaltic pump and to the perfusion cannula. The coronary effluent from the 10 minute prewash was discarded.

After the preliminary 10 minute prewash the heart was transferred to the recirculating system containing approximately 80-90ml of buffer.

The perfusion pressure was adjusted by controlling the output of the Masterflex rotary recirculation pump (model 7014) which was connected to a calibrated mercury manometer. For these experiments 60mmHg perfusion pressure at the inherent heart rate was chosen as the basal condition, because Neely et al (21) have suggested that 60mmHg is sufficient to maintain coronary flow, substrate and oxygen delivery in the isolated perfused rat heart.

For the experimental conditions, one hour of perfusion at 60mmHg was considered the base line condition, or low workload since any pressure below that could be detrimental to the heart (21). An increased perfusion pressure invokes an increased amount of work (19,20,27,43) and hemodynamic performance (19,20,43). In this study 120mmHg was chosen as the most severe workload, since previous results (21,43) have indicated that ventricular performance was well maintained at this pressure. A 90mmHg perfusion pressure was chosen as a moderate workload condition, being equally above and below the selected low and high perfusion pressures.

Hearts were perfused at each pressure for 15, 30, 45 and 60 minutes. At the end of each perfusion the heart was cut from the cannula, wrapped in tin foil, frozen in liquid nitrogen and stored in a Canlab Jewett Refrigerator (model CTF-1) at -40°C.

Pressure Measurements and Recordings

At the start of the prewash, the onset of the experimental perfusion and at 15 minute intervals thereafter, 10 second pressure changes were recorded on a Hewlett-Packard 1500-B electrocardiograph. In addition, the aortic pressure was continuously monitored by connecting a Statham P-23Db physiological pressure transducer (coupled to a Hewlett-Packard 350-1100C carrier preamplifier) to a Hewlett-Packard Sanborn 780-6A Viso Scope (sweep rate

of 25mm/second). Hearts that were arrhythmic or were incapable of developing and maintaining aortic pressures equal to, or greater than, the perfusion pressure, were discarded. Heart rates were determined from the pressure recordings.

Tissue Preparation and Assay

For glycogen determinations a portion of the frozen apex (200-400mg) was removed, placed into a previously weighed test tube containing 2ml of 30% KOH saturated with Na_2SO_4, reweighed to determine the tissue weight (Sartorius Micro Balance, type 2462) and put into a boiling water bath until a homogeneous solution was obtained. The test tubes were cooled in ice and 95% ethanol added (1.1 to 1.2 volume) and mixed thoroughly. The tubes were then placed in the refrigerator overnight to allow precipitation of the glycogen. The samples were centrifuged in an I.E.C. International Centrifuge (model HT) at 3000g for 15 minutes at room temperature. The supernatent was carefully aspirated and the precipitate dissolved up to a 2ml volume of double distilled deionized water. Aliquots (.2ml) were removed for glycogen determinations by the phenol-sulphuric acid technique of Lo et al (12) (Appendix A). Standard glycogen solutions were prepared from liver glycogen (.050mg/10ml double distilled deionized water) and appropriate aliquots removed for the glycogen standard curve (Appendix A,

Figure 2). The remaining tissue, exclusive of atria and aorta, was weighed to determine the wet weight to dry weight ratio. The tissue was dried in a Precision Scientific Freas Dryer (model 845) for 4 days at 70°F.

The rates of glycogen depletion (ug/gm/min) were determined using a Hewlett-Packard 65 Calculator, by averaging the 5 glycogen values at each of the 15 minute intervals and calculating the mean change in glycogen levels every 15 minutes. This mean change in glycogen levels (ug/gm) was then divided by 15 minutes, to give the rate of glycogen depletion in ug/gm/minute of perfusion.

Analysis of Data

The IBM 360 Computer, at the University of Windsor, was used for all statistical analysis of the data. To analyze for significant changes in glycogen levels over time and between conditions the program Procedure ANOVA (Appendix B) for independent samples using the least significant difference technique was selected by the computer personnel.

Because the results of the analysis of variance and calculations of the rates of glycogen depletion failed to indicate the patterns of glycogen depletion, a regression on dummy variables was done to give an indication of the best fitting curves for each of the 3 conditions.

CHAPTER III

RESULTS

Effect of Increased Perfusion Pressure on Heart Rate and Water Content

A consistent heart rate and degree of edema are critical in the comparison of the metabolic and mechanical properties of the perfused heart. The results of the measurement of heart rate and edema during 1 hour of perfusion at 60, 90 or 120mmHg are presented in Tables 1 and 2.

Under a constant perfusion pressure of 60, 90 or 120mmHg, the rate of contraction was constant. However, as the perfusion pressure was increased, there was a slight increase in the contraction rate.

The degree of edema was recorded to assess the effect of perfusion on the tissue water content. The amount of edema was similar for all of the experimental conditions following 45 and 60 minutes of perfusion.

Effect of Increased Perfusion Pressures on the Pattern of Glycogen Depletion

The levels of myocardial glycogen in the intact animal and during the experimental procedures are shown in Figure 3. The results show that the glycogen levels at the start of the perfusions were similar to those

TABLE 1

EFFECT OF PERFUSION PRESSURE ON RATES OF CONTRACTION ±S.D.

TIME (min.)	Perfusion Pressure mmHg 60	90	120
End of Prewash	244±9 (30)		
Start of Experimental Condition	244±9 (11)	256±16 (14)	268±9 (15)
15	239±10 (14)	258±11 (12)	269±8 (15)
30	234±10 (12)	249±13 (15)	272±5 (15)
45	225±10 (10)	246±8 (10)	261±8 (10)
60	230±17 (5)	243±13 (5)	271±5 (5)

TABLE 2

EFFECT OF PERFUSION PRESSURE ON DRY WEIGHT:WET WEIGHT RATIO OF RAT VENTRICLES AEROBICALLY PERFUSED

Perfusion Pressure (mmHg)	Percent Dry Weight:Wet Weight
In Vivo	26.66 (6)
60	20.85 (10)
90	18.54 (10)
120	19.32 (10)

The 60, 90 and 120mmHg values are the mean percentage of hearts perfused for 45 and 60 minutes.

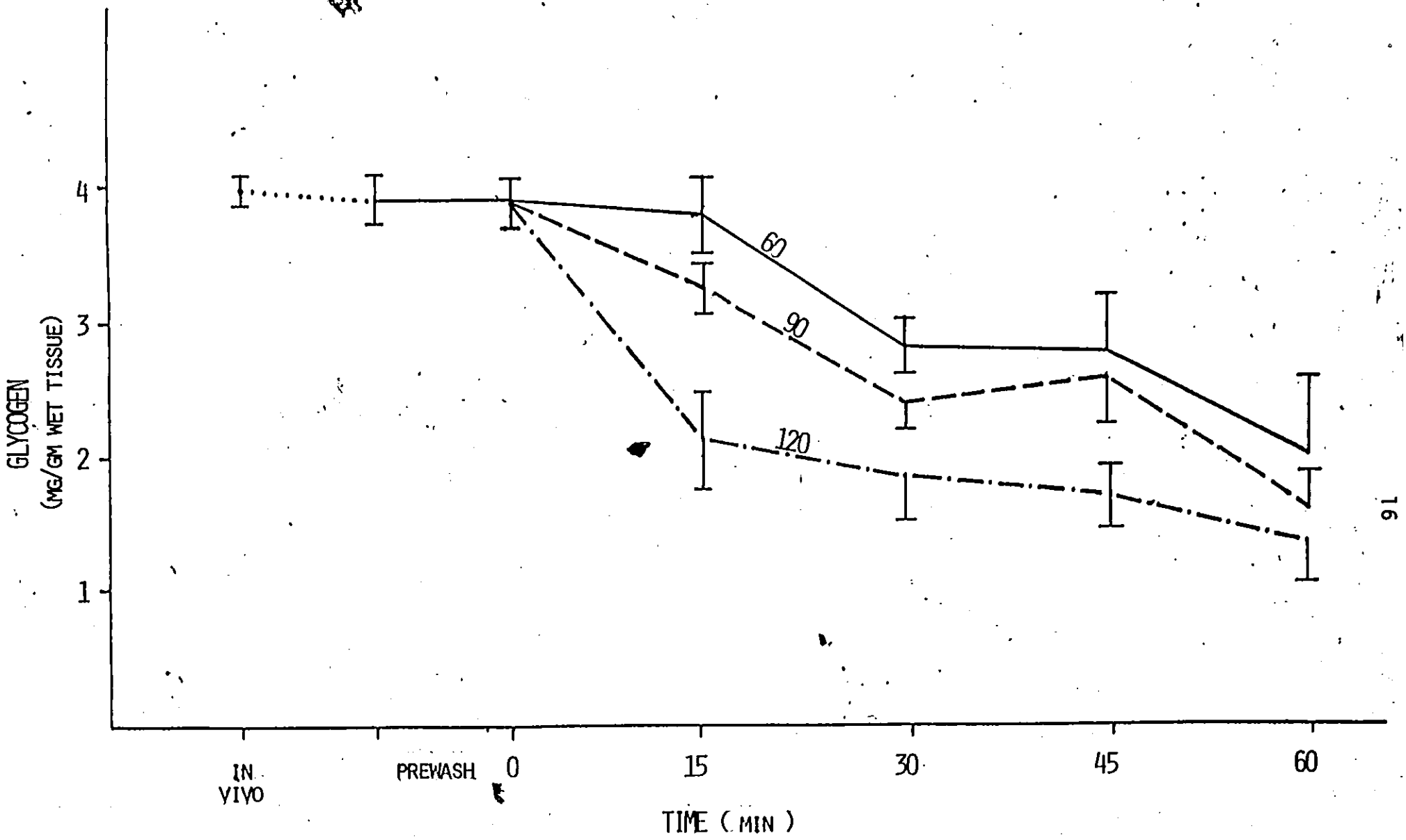

FIGURE 3. GLYCOGEN CONCENTRATIONS ± S.D. PRIOR TO AND DURING ONE HOUR OF AEROBIC PERFUSION AT 60, 90 AND 120 mmHg AORTIC PRESSURE

recorded in vivo. Thus, the procedures prior to the experimental conditions did not affect the initial levels of glycogen. Similar glycogen levels have been reported by others (1,8,10,34,35,37,40).

The results of an increased perfusion pressure on the patterns and rates of glycogen depletion are seen in Figure 3 and Table 3. It can be seen that under 3 workloads, 3 different patterns of glycogen depletion occur.

The results also indicate that only during the first 15 minutes of perfusion was glycogen depletion directly related to the intensity of the work. During the next 15 minutes, however, 60mmHg showed the highest rate of glycogen depletion followed by 90 and 120mmHg respectively. The patterns in which glycogen was utilized over the remaining 30 minutes was similar in all conditions, however, the levels of glycogen within the patterns were not the same.

More specific examination of the results (Table 3) indicated that at the low perfusion pressure of 60mmHg the rate of glycogen depletion was slow (6.9 ug/gm/min) during the initial 15 minutes of perfusion, resulting in an insignificant ($P > .05$) decrease in glycogen levels. However, during the next 15 minutes there was a 27% decrease ($P < .01$) in glycogen levels. The rate of glycogen depletion was low and not statistically significant during 30-45 minutes of perfusion but rose markedly between 45-60

TABLE 3

EFFECT OF PERFUSION PRESSURE ON THE RATES OF GLYCOGEN DEPLETION

Perfusion Time	Perfusion Pressure mmHg		
	60	90	120
0-15	6.9	43.2	118.6
15-30	67.5	57.5	14:7
30-45	0.1	15.0*	13.1
45-60	52.5	68.4	22.0

units = ug/gm/minute

*synthesis of glycogen

Values are mean of 5 animals.

minutes, significantly decreasing (P .01) the glycogen concentration.

In hearts working against 90mmHg perfusion pressure, a high rate of glycogen breakdown significantly decreased (P .01) glycogen levels at both 15 and 30 minutes of perfusion. Between 30-45 minutes, glycogen levels were not statistically altered (P .05), while during the last 15 minutes, the rate of glycogen breakdown increased, significantly decreasing (P .01) the levels of glycogen.

At 120mmHg, 40% of the total myocardial glycogen was utilized (P .01) during the first 15 minutes of work. Following this, glycogen levels decreased slowly until 45-60 minutes, where glycogen depletion rose, significantly decreasing (P .01) myocardial glycogen stores.

The results of the regression can be seen in Appendix B, and indicate that the pattern of glycogen depletion under any workload followed no predictable pattern of depletion.

CHAPTER IV

DISCUSSION

An interpretation of results using the perfused heart system must be based on the assumptions that the heart rates were constant and that water accumulation and subsequent dilution is equal for all conditions.

The recorded contraction rates are in agreement with those of Taylor and Cerny (43) and Neely et al (21), indicating a slightly increased rate of contraction was associated with an increased perfusion pressure, but that at any given pressure, rates were constant during 1 hour of perfusion.

The degree of edema, in this and earlier studies (29,38) indicate an approximate 5% water accumulation during perfusion, suggesting that a decrease in the glycogen concentration was not related to an increase in the wet weight of the tissue. The significance of edema on the mechanical and metabolic performance of the heart is yet to be resolved. With edema being relatively constant, it may be assumed, however, that all of the hearts were being affected to the same relative degree during 1 hour of perfusion.

The results are in agreement with the literature

(18-20,22,24,27) that the rate of glycogen depletion was increased as the workload was raised and that the patterns of glycogen depletion were different for each workload.

Glycogenolysis is controlled primarily by the activity of the enzyme phosphorylase (17,23,26,27), which is activated by 5'-AMP. At rest and low workloads, however, active inhibition of phosphorylase activation by high levels of glucose-6-phosphate (G-6-P) (16,23,26,27) and adenosine triphosphate (ATP) (16,23,26,27) prevent the breakdown of glycogen.

As reported earlier, Neely et al (18,19,24,27) have suggested that decreases in G-6-P and increases in inorganic phosphate could account for phosphorylase 'b' activation, stimulating glycogen breakdown; rates being faster at the higher workloads. Changes in the pattern of glycogen depletion would then mostly indicate changes in the concentration of G-6-P.

The present results indicate that work induced glycogen breakdown during the first 15 minutes was directly related to workload, as an increase in perfusion pressure to 90 or 120mmHg stimulated glycogen breakdown, with no significant depletion at 60mmHg.

In addition, the 60mmHg glycogen depletion pattern was in direct contradiction to those of Kreisberg (11), Morgan (15) and Williamson (47,48), reporting no significant glycogen change while perfusing with 5mM glucose for 30, 60 and 75 minutes, respectively.

The results of 60mmHg perfusion pressure during the first 15 minutes agree with the work of others (11,15,47,48) that physiological concentrations of exogenous glucose are able to maintain glycogen levels. Perhaps, intracellular G-6-P levels were unaltered, thus inhibiting glycogen breakdown, but as the workload increased to 90 or 120mmHg, the increased energy demand may have decreased the G-6-P levels, stimulating glycogen breakdown.

Between 15-30 minutes of perfusion, 60mmHg resulted in the highest glycogen depletion while 120mmHg perfusion pressure caused the lowest. The sudden depletion at 60mmHg might be explained by a possible depletion of endogenous lipids (5,6,18,26,41), stimulating glycogen utilization as an alternative substrate. On the other hand, the decreased glycogen utilization at 120mmHg may have been due to an adjustment of aerobic metabolism to supply the required ATP, through utilization of other available alternative substrates (i.e., glucose, lactate, lipids), sparing glycogen stores.

At a perfusion pressure of 90mmHg, the rate of glycogen depletion remained relatively constant and might be due to a continued low level of G-6-P stimulating glycogenolysis from 15-30 minutes or due to a depletion of endogenous lipids.

During the last 30 minutes of perfusion the 3 patterns of glycogen depletion were similar, in that rates

were decelerated from 30-45 minutes and were accelerated from 45-60 minutes. The insignificant decrease in glycogen levels seen between 30-45 minutes at all pressures may have been due to either an increased exogenous glucose oxidation associated with prolonged perfusions (3,5,22,31, 44) or the utilization of lactate, formed during the preceding periods of perfusion.

The glycogen depletion between 45-60 minutes could be caused by a fall in G-6-P levels, stimulating glycogen breakdown. This fall in G-6-P may be a result of a lowering of the exogenous glucose concentration, therefore, limiting its own transport and uptake. Neely et al (28) have indicated that a 5mM glucose perfusate was inadequate to saturate glucose transport and Opie et al (32) suggesting low levels of exogenous glucose were inadequate to maintain glycogen levels. This significant depletion of glycogen levels also suggests that glycogen utilization was not controlled by its own levels, but by the activity of the enzyme phosphorylase and the availability of alternative substrates.

In conclusion, it is apparent that the patterns of glycogen depletion under the 3 workloads, followed no distinct pattern of depletion, but a combination of patterns, being controlled initially by the workload imposed. Following this, glycogen depletion may be dependent on the availability of alternative substrates, and the level and activity of the enzyme, phosphorylase.

CHAPTER V

SUMMARY AND CONCLUSION

Experimental Design

It was hypothesized that increasing the workload on the isolated heart, perfused with physiological glucose concentrations, would increase glycogen utilization and that under 3 perfusion pressures, 3 different patterns of glycogen utilization would occur.

To test this hypothesis, myocardial glycogen levels were measured at 15 minute intervals over 1 hour of perfusion, at 60, 90 and 120mmHg perfusion pressure, using a modified Langendorff perfusion apparatus with Krebs-Henseleit bicarbonate buffer, containing 5.5mM glucose. A standard 10 minute preliminary prewash, preceded the experimental perfusions of 1 hour.

Results and Discussion

Results of the analysis of variance of the data for between conditions and within conditions indicate that there were significant differences in the levels of glycogen at various times, for the 3 conditions. The 120mmHg condition resulted in the most significant initial decrease in glycogen levels, followed by 90 and 60mmHg respectively. It was suggested that during the initial

15 minutes, glycogen depletion was a direct result of the workload, the higher the perfusion pressure, the greater the decrease in glucose-6-phosphate, stimulating glycogen breakdown.

The next 15 minutes of perfusion indicated that glycogen depletion was greatest at 60mmHg, with smaller decreases at 90 and 120mmHg. The significant change in glycogen levels seen at 60mmHg may be the result of a depletion of oxidizable endogenous lipids, forcing the heart to use glycogen as an alternative energy substrate. The continued depletion of glycogen stores seen at 90mmHg may indicate that glucose-6-phosphate levels were still low. The decrease in glycogen breakdown at 120mmHg might be indicating that aerobic metabolism was supplying the necessary ATP, utilizing alternative substrate (endogenous lipids, glucose, lactate), thereby possibly inhibiting glycogen breakdown. The insignificant change in glycogen levels between 30-45 minutes, under all conditions, may have been due to the utilization of other substrates by the tissue thus sparing glycogen.

The statistically significant decrease in glycogen levels seen during the last 15 minutes at all 3 perfusion pressures may have been caused by a fall in glucose-6-phosphate as a result of limited glucose transport with the decrease in the exogenous glucose concentration.

Conclusion

As was hypothesized, an increase in perfusion pressure resulted in an increase in glycogen utilization and that under 3 workloads, 3 different patterns of glycogen depletion did exist.

The patterns of glycogen depletion for each workload, however, were not as hypothesized, indicating the need for further research concerning the mechanisms regulating glycogen breakdown seen in the present experiment.

Suggestions for Further Study

The results of the present experiment might be improved by increasing the number of rats, use of the whole heart for glycogen determinations and by using electrical pacing to eliminate the effect of an elevated heart rate associated with an increased perfusion pressure on glycogen metabolism.

A repeat of the experiments, measuring not only glycogen, but exogenous glucose, endogenous lipids, lactate levels and the glycolytic enzymes, particularly phosphorylase would be helpful in understanding the factors controlling glycogen breakdown.

Increasing the number of times during the 1 hour of perfusion where glycogen levels are determined may also give some insight as to metabolic interplay affecting glycogen depletion.

It would also seem to be justified to do some experiments using electrical pacing of hearts, as unpublished data from this lab indicate that heart rate may increase glycogen utilization significantly. Experiments using the working heart system and comparing the results to those of the non-working preparation might also prove to be interesting.

To study the effect of elevated glycogen stores (eg., through training or fasting) on glycogen depletion and attempt to distinguish whether free or bound glycogen was being used and when these glycogen fractions were used by the tissue, might also give a better understanding of glycogen breakdown.

BIBLIOGRAPHY

[1]Barnard, R. J. and A. T. Thorstensson. "Effect of Exhaustive Exercise on the Rat Heart." In, Metabolic Adaptation to Prolonged Physical Exercise, edited by H. Howald and J. R. Poortmans. pp. 431-437. Birkhauser Verlag Basel, Switzerland, 1975.

[2]Blount, D. H. and D. K. Meyer. "Effect of Cardiac Work on Glycogen Fractions of the Heart." Am. J. Physiol. 197:1013-1016, 1959.

[3]Chain, E. B., K. R. L. Mansford and L. H. Opie. "Effects of Insulin on the Pattern of Glucose Metabolism in the Perfused Working and Langendorff Heart of Normal and Insulin-Deficient Rats." Biomech. J. 115:537-546, 1969.

[4]Crass, M. F. III, E. S. McCaskill, and J. C. Shipp. "Glucose-free Fatty Acid Interactions in the Working Heart." J. Appl. Physiol. 29:87-91, 1970.

[5]Crass, M. F. III, E. S. McCaskill and J. C. Shipp. "Effect of Pressure Development on Glucose and Palmitate Metabolism in Perfused Heart." Am. J. Physiol. 216:1569-1576, 1969.

[6]Crass, M. F. III, E. S. McCaskill, J. C. Shipp, and V. K. Murthy. "Metabolism of Endogenous Lipids in Cardiac Muscle: Effect of Pressure Development." Am. J. Physiol. 220:428-435, 1971.

[7]Dhalla, N. S., R. F. Matoushek, C. N. Sun and R. E. Olson. "Metabolic, Ultrastructural, and Mechanical Changes in the Isolated Rat Heart Perfused with Aerobic Medium in the Absence or Presence of Glucose." Can. J. Physiol. Pharmacol. 51:590-603, 1973.

[8]Evans, G. "The Glycogen Content of the Rat Heart." J. Physiol., London 82:468-480, 1934.

[9]Gartner, S. L. and G. V. Vahourny. "Endogenous Triglyceride and Glycogen in Perfused Rat Hearts." Proc. Soc. Exp. Biol. Med. 143:556-560, 1973.

[10]Judd, W. T. and J. L. Poland. "Myocardial Glycogen Changes with Exercise." Proc. Soc. Exp. Biol. Med. 140:955-957, 1972.

[11]Kreisberg, R. A. and J. R. Williamson. "Metabolic Effects of Glucagon in the Perfused Rat Heart." Am. J. Physiol. 207:721-727, 1964.

[12]Lo, S., J. C. Russell and A. W. Taylor. "Determination of Glycogen in Small Tissue Samples." J. Appl. Physiol. 28:234-236, 1970.

[13]Matoushek, R. N., S. Dhalla and R. E. Olson. "Effect of Perfusion with Substrate Free Medium on Glycolytic and Citric Acid Cycle Intermediates in Rat Heart." Fed. Proc. 27:303, 1968.

[14]Morgan, H. E., M. J. Henderson, D. M. Regen and C. R. Park. "Regulation of Glucose Uptake in Muscle. I. The Effects of Insulin and Anoxia on Glucose Transport and Phosphorylation in the Isolated Perfused Heart of Normal Rats." J. Biol. Chem. 236:253-261, 1961.

[15]Morgan, H. E., J. R. Neely, R. E. Wood and C. Liebecq, H. Liebermeister and C. R. Park. "Factors Affecting Glucose Transport in Heart Muscle and Erythrocytes." Fed. Proc. 24:1040-1045, 1965.

[16]Morgan, H. E. and A. Parmeggiani. "Regulation of Glycogenolysis in Muscle. III. Control of Muscle Glycogen Phosphorylase Activity." J. Biol. Chem. 239:2440-2445, 1964.

[17]Morgan, H. E. and A. Parmeggiani. "Regulation of Glycogenolysis in Muscle. II. Control of Glycogen Phosphorylase Reaction in Isolated Perfused Heart." J. Biol. Chem. 239:2435-2439, 1964.

[18]Neely, J. R., R. H. Bowan and H. E. Morgan. "Effects of Ventricular Pressure Development and Palmitate on Glucose Transport." Am. J. Physiol. 216:804-811, 1969.

[19]Neely, J. R., R. H. Bowman and H. E. Morgan. "Conservation of Glycogen in the Perfused Rat Heart Developing Intraventricular Pressure. In, Control of Glycogen Metabolism, edited by W. J. Whelan. pp. 49-64. Universitetsforlaget, Oslo, 1968.

[20]Neely, J. R., R. M. Denton, P. J. England and P. J. Randle. "The Effects of Increased Heart Work on the Tricarboxylate Cycle and Its Interactions with Glycolysis in the Perfused Rat Heart." Biochem. J. 128:147-159, 1972.

[21]Neely, J. R., H. Liebermiester, E. J. Battersby and H. E. Morgan. "Effect of Pressure Development on Oxygen Consumption by Isolated Rat Heart." Am. J. Physiol. 212:804-814, 1967.

[22]Neely, J. R., H. Liebermiester and H. E. Morgan. "Effect of Pressure Development on Membrane and Transport of Glucose in Isolated Rat Heart." Am. J. Physiol. 212:815-822, 1967.

[23]Neely, J. R. and H. E. Morgan. "Relationship Between Carbohydrate and Lipid Metabolism and Energy Balance of Heart Muscle." Am. Rev. Physiol. 36:413-459, 1974.

[24]Neely, J. R. and H. E. Morgan. "Regulation of Glycogenolysis in Hearts Developing Intraventricular Pressure." Fed. Proc. 27:580, 1968.

[25]Neely, J. R. and M. J. Rovetto. "Techniques for Perfusing Isolated Rat Hearts." Methods in Enzymology 39:43, 1975.

[26]Neely, J. R., M. J. Rovetto and J. F. Oram. "Myocardial Utilization of Carbohydrates and Lipids." Prog. Cardiovasc. Dis. 15:289-329, 1972.

[27]Neely, J. R., C. F. Whitfield and H. E. Morgan. "Regulation of Glycogenolysis in Hearts: Effects of Pressure Development, Glucose, and FFA." Am. J. Physiol. 219:1083-1088, 1970.

[28]Neely, J. R., K. M. Whitmer and S. Mochizuki. "Effects of Mechanical Activity and Hormones on Myocardial Glucose and Fatty Acid Utilization." Circ. Res. Supp. 1, 38:22-30, 1976.

[29]Olson, R. E. and R. J. Hoeschen. "Utilization of Endogenous Lipid by the Isolated Perfused Rat Heart." Biochem. J. 103:796-801, 1967.

[30]Opie, L. H., J. R. Evans and J. C. Shipp. "Effect of Fasting on Glucose and Palmitate Metabolism of Perfused Rat Heart." Am. J. Physiol. 205:1203-1208, 1963.

[31]Opie, L. H., K. R. L. Mansford and P. Owen. "Effects of Increased Heart Work on Glycolysis and Adenine Nucleotides in the Perfused Heart of Normal and Diabetic Rats." Biochem. J. 124:475-490, 1971.

[32]Opie, L. H., J. C. Shipp, J. R. Evans and B. Leboeuf. "Metabolism of Glucose-U-C'4 in Perfused Rat Heart." Am. J. Physiol. 203:839-843, 1962.

[33]Poland, J. L. and D. H. Blount. "Glycogen Depletion in Rat Ventricles During Graded Exercise." Proc. Soc. Exp. Biol. Med. 121:560-562, 1966.

[34]Poland, J. L., T. D. Myers, R. J. Witorsch and R. B. Brandt. "Plasma Corticosterone and Cardiac Glycogen Levels in Rats After Exercise." Proc. Soc. Exp. Biol. Med. 150:148-150, 1975.

[35]Poland, J. L. and D. A. Trauner. "Adrenal Influence on the Supercompensation of Cardiac Glycogen Following Exercise." Am. J. Physiol. 224:540-542, 1973.

[36]Reitman, J., K. M. Baldwin and J. O. Holloszy. "Intramuscular Triglyceride Utilization by Red, White, and Intermediate Skeletal Muscle and Heart During Exhausting Exercise." Proc. Soc. Exp. Biol. Med. 142:628-631, 1973.

[37]Russell, A.and W. Bloom. "Hormonal Control of Glycogen in the Heart and Other Tissues in Rats." Endocrinology 58:83-94, 1956.

[38]Scheuer, J. and S. W. Stezoski. "Protective Role of Increased Myocardial Glycogen Stores in Cardiac Anoxia in the Rat." Circ. Res. 27:835-849, 1970.

[39]Scheuer, J. and S. W. Stezoski. "A Protective Effect of Increased Glycogen Stores in Cardiac Anoxia." J. Lab. Clin. Med. 74:1007-1008, 1969.

[40]Segel, L. D., A. Chung, D. T. Mason and E. A. Amstordam. "Cardiac Glycogen in Long-Evans Rats: Diurnal Pattern and Response to Exercise." Am. J. Physiol. 229:398-401, 1975.

[41]Shipp, J. C., O. E. Matos, H. Knizley and L. E. Crevasse. "CO_2 Formed From Endogenous and Exogenous Substrates in Perfused Rat Heart." Am. J. Physiol. 207:1231-1236, 1964.

[42]Shipp, J. C., L. H. Opie and D. Shalloner. "Fatty Acid and Glucose Metabolism in the Perfused Heart." Nature 189:1018-1019, 1961.

[43]Taylor, P. B. and F. C. Cerny. "Evaluation of the Isolated Paced Rat Heart." J. Appl. Physiol. 41:000, 1976. (in press)

[44]Vahourny, G. V., J. Liljenquist, R. Wilson, A. Liao and S. L. Rodis. "Myocardial Metabolism. III. Myocardial Intermediates and Substrate Oxidation During In Vitro Perfusion of Palmitate and Glucose." Arch. Biochem. Biophys. 125:809-816, 1968.

[45]White, A., P. Handler and E. L. Smith. Principles of Biochemistry, McGraw-Hill Book Company, New York, 1973.

[46]Williamson, J. R. "Glycolytic Control Mechanisms. I. Inhibition of Glycolysis by Acetate and Pyruvate in the Isolated Perfused Rat Heart." J. Biol. Chem. 240:2308-2321, 1965.

[47]Williamson, J. R. "Effects of Insulin and Diet on the Metabolism of L(+)-Lactate and Glucose by the Perfused Rat Heart." Biochem. J. 83:377-383, 1962.

[48]Williamson, J. R. and H. A. Krebs. "Acetoacetate as Fuel of Respiration in the Perfused Rat Heart." Biochem. J. 80:540-547, 1961.

APPENDIX A

GLYCOGEN DETERMINATION

Glycogen Determination

Phenol-Sulphuric Acid Technique (12)

1. .2ml of the glycogen solution were pipetted into a test tube and 0.8ml of double distilled deionized water were added.
2. 1 milliliter of 5% phenol solution was added to the above.
3. 5 milliliters of 96% H_2SO_4 were added rapidly (10-20 seconds), the stream of acid being directed against the liquid surface rather than against the side of the test tube, to ensure good mixing.
4. The tubes were then allowed to stand for 10 minutes.
5. They were shaken and placed for 20 minutes in a water bath at 25°-30°C, before readings were taken.
6. Blanks were prepared by using 1.0ml of double distilled deionized water instead of the glycogen solution.
7. The absorbance was read on a Bausch and Lomb Spectronic 20 at 490 mu.
8. All tests were carried out in triplicate to minimize errors.

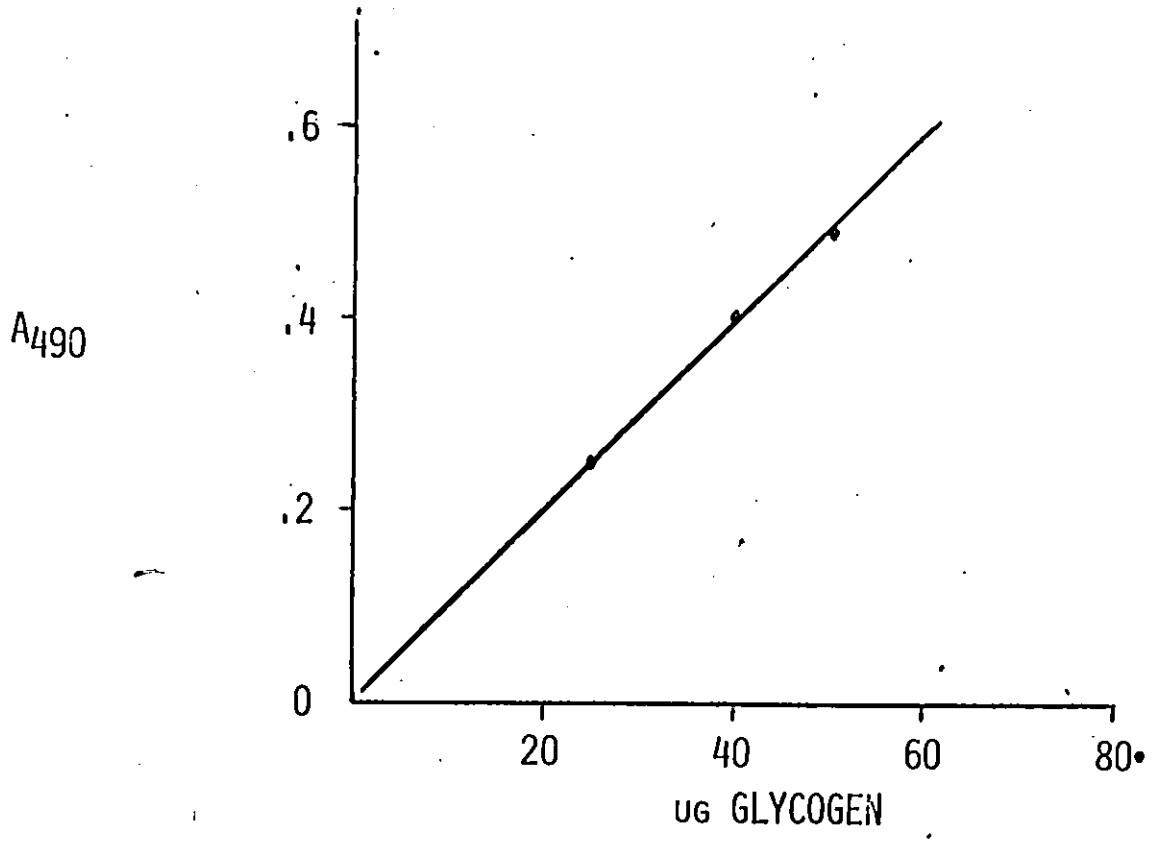

FIGURE 2. STANDARD CURVE FOR PHENOL-SULPHURIC ACID-GLYCOGEN REACTION. EACH POINT IS MEAN OF THREE DETERMINATIONS.

APPENDIX B

ANALYSIS OF DATA

```
DATA ; INPUT COND $ 1-5 T0 7-11 T15 13-17 T30 19-23 T45 25-29 T60 31-35 ;
DROP T0 T15 T30 T45 T60 ;
TIME=0 ; X=T0 ; OUTPUT ;
TIME=15 ; X=T15 ; OUTPUT ;
TIME=30 ; X=T30 ; OUTPUT ;
TIME=45 ; X=T45 ; OUTPUT ;
TIME=60 ; X=T60 ; OUTPUT ;
CARDS
```

75 OBSERVATIONS IN DATA SET DATA0001 3 VARIABLES

```
PROC ANOVA ; CLASSES COND TIME ;
MEANS COND TIME COND*TIME ;
MODEL X=COND|TIME ;
POOL 'ERROR2' COND*TIME RESIDUAL/COND TIME ;
TEST COND TIME COND*TIME BY RESIDUAL ;
TEST COND TIME BY 'ERROR2' ;
```


TABLE 4

MEANS

COND	TIME	N	X
60 MSO	0	5	3.88120000
60 MSO	15	5	3.77720000
60 MSO	30	5	2.76400000
60 MSO	45	5	2.76200000
60 MSO	60	5	1.97340000
90 MSO	0	5	3.88120000
90 MSO	15	5	3.23240000
90 MSO	30	5	2.36880000
90 MSO	45	5	2.59400000
90 MSO	60	5	1.56740000
99 MSO	0	5	3.88120000
99 MSO	15	5	2.10160000
99 MSO	30	5	1.87980000
99 MSO	45	5	1.68300000
99 MSO	60	5	1.35160000

ANALYSIS OF VARIANCE FOR VARIABLE X MEAN 2.64658667 C.V. 12.5410259 %

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	LSD .01	LSD .05	DIVISOR
COND	2	[illegible]	[illegible]			
TIME	4	43.[illegible]4994	10.[illegible]3748			
COND*TIME	8	4.3049127	0.5381141			
ERROR2	60	10.[illegible]115	0.1688211	0.[illegible]0098	0.[illegible]0351	25
RESIDUAL	60	6.[illegible]188	0.1105153			
CORRECTED TOTAL	74	[illegible]	[illegible]			

```
PROC REGR ; CLASS TIME ;
MODEL X=LINE QUAD CUBIC QUART TIME ;
BY COND ;
```

STATISTICAL ANALYSIS SYSTEM
COND=60MSO

ANALYSIS OF VARIANCE TABLE , REGRESSION COEFFICIENTS , AND STATISTICS OF FIT FOR DEPENDENT VARIABLE X

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB > F	R-SQUARE	C.V.
REGRESSION	4	12.70910336	3.17727584	21.68209	0.0001	0.81239744	12.63605 %
ERROR	20	2.93484480	0.14674224				
CORRECTED TOTAL	24	15.64394816				STD DEV	X MEAN
						0.38306950	3.03155

SOURCE	DF	SEQUENTIAL SS	F VALUE	PROB > F	PARTIAL SS	F VALUE	PROB > F
LINE	1	11.66831432	79.51572	0.0001	11.66831432	79.51572	0.0001
QUAD	1	0.04577286	0.31193	0.5888	0.04577286	0.31193	0.5888
CUBIC	1	0.00751538	0.05121	0.8177	0.00751538	0.05121	0.8177
QUART	1	0.98750080	6.72949	0.0165	0.98750080	6.72949	0.0165
TIME	0	0.0	0.0	1.0000	0.0	0.0	1.0000

STATISTICAL ANALYSIS SYSTEM
COND=90MSO

ANALYSIS OF VARIANCE TABLE , REGRESSION COEFFICIENTS , AND STATISTICS OF FIT FOR DEPENDENT VARIABLE X

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB > F	R-SQUARE	C.V.
REGRESSION	4	15.39129856	3.84782464	39.00094	0.0001	0.88636606	11.51078 %
ERROR	20	1.97319600	0.09865980				
CORRECTED TOTAL	24	17.36449456				STD DEV	X MEAN
						0.31410158	2.72876

SOURCE	DF	SEQUENTIAL SS	F VALUE	PROB > F	PARTIAL SS	F VALUE	PROB > F
LINE	1	13.86537800	140.53726	0.0001	13.86537800	140.53726	0.0001
QUAD	1	0.03965080	0.40189	0.5398	0.03965080	0.40189	0.5398
CUBIC	1	0.53768450	5.44988	0.0285	0.53768450	5.44988	0.0285
QUART	1	0.94858526	9.61471	0.0057	0.94858526	9.61471	0.0057
TIME	0	0.0	0.0	1.0000	0.0	0.0	1.0000

STATISTICAL ANALYSIS SYSTEM
COND=99MSO

ANALYSIS OF VARIANCE TABLE , REGRESSION COEFFICIENTS , AND STATISTICS OF FIT FOR DEPENDENT VARIABLE X

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB > F	R-SQUARE	C.V.
REGRESSION	4	19.61801016	4.90450254	86.93383	0.0001	0.91926569	13.66689 %
ERROR	20	1.72287800	0.08614390				
CORRECTED TOTAL	24	21.34088816				STD DEV	X MEAN
						0.29350281	2.17944

SOURCE	DF	SEQUENTIAL SS	F VALUE	PROB > F	PARTIAL SS	F VALUE	PROB > F
LINE	1	15.00314642	174.16377	0.0001	15.00314642	174.16377	0.0001
QUAD	1	3.04804356	35.38339	0.0001	3.04804356	35.38339	0.0001
CUBIC	1	1.41100000	16.00361	0.0006	1.41100000	16.00361	0.0006
QUART	1	0.13469130	1.56306	0.2227	0.13469130	1.56306	0.2227
TIME	0	0.0	0.0	1.0000	0.0	0.0	1.0000

APPENDIX C

INDIVIDUAL DATA

TABLE 6

GLYCOGEN CONCENTRATIONS (mg/gm wet tissue) IN VIVO, DURING CANNULATION AND AT THE END OF PREWASH

	In Vivo	Cannulation	End of Prewash
	3.912	4.100	3.755
	4.064	3.805	3.850
	4.033	4.097	4.174
	3.769	3.752	3.820
	3.977	3.803	3.807
Mean	3.951	3.911	3.881
S.D.	.117	.172	.167
S.E.M.	.052	.076	.074

TABLE 7

GLYCOGEN CONCENTRATIONS (mg/gm wet tissue)

DURING 1 HOUR OF PERFUSION AT 60mmHg

	Time of Perfusion				
	0	15	30	45	60
	3.755	3.335	2.423	3.167	1.979
	3.850	4.174	2.753	3.060	2.552
	4.170	3.720	2.962	2.116	2.524
	3.820	3.850	2.986	2.375	1.264
	3.807	3.809	2.696	3.092	1.548
Mean	3.881	3.777	2.764	2.762	1.973
S.D.	.167	.301	.228	.481	.574
S.E.M.	.074	.134	.102	.215	.257

TABLE 8

GLYCOGEN CONCENTRATIONS (mg/gm wet tissue)

DURING 1 HOUR OF PERFUSION AT 90mmHg

	Time of Perfusion				
	0	15	30	45	60
	3.755	3.343	1.841	2.125	1.080
	3.850	3.401	2.543	2.958	1.866
	4.174	2.929	2.738	2.297	1.588
	3.820	3.200	2.387	2.340	1.631
	3.807	3.289	2.335	3.250	1.673
Mean	3.881	3.232	2.368	2.594	1.567
S.D.	.167	.185	.334	.483	.483
S.E.M.	.074	.082	.149	.216	.216

TABLE 9

GLYCOGEN CONCENTRATIONS (mg/gm wet tissue)
DURING 1 HOUR OF PERFUSION AT 120mmHg

	Time of Perfusion				
	0	15	30	45	60
	3.755	2.266	1.541	1.970	.996
	3.850	2.068	1.734	1.851	1.642
	3.820	1.801	1.666	1.531	1.053
	4.174	2.665	2.163	1.388	1.535
	3.807	1.708	2.294	1.675	1.532
Mean	3.881	2.101	1.879	1.683	1.351
S.D.	.167	.384	.329	.234	.302
S.E.M.	.074	.171	.147	.105	.135

VITA AUCTORIS

Name: Kenji Alan Kenno

BIOGRAPHICAL DATA

Place and Date of Birth: Fort William, Ontario
May 25, 1951

Education:

B.P.H.E. Lakehead University
1974

M.H.K. University of Windsor
1976